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EXAMINER	
BERTOGLIO, VALARIE E	

ART UNIT	PAPER NUMBER
1632	

DATE MAILED: 09/08/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/863,528	NEBERT, DANIEL W.	
	Examiner	Art Unit	
	Valarie Bertoglio	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-37* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-37* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *22 May 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *07/30/2002*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

The preliminary amendments received 02/14/2002 and 04/02/2004 have been entered. Claims 1-37 are pending and under consideration in the instant office action.

Specification

Table 1 has been amended to include sequence identifiers. However, the various and different sequences all have the same SEQ ID NO:1 sequence identifier. The sequence listing assigns different sequence identifiers to each sequence listed in Table 1.

Claim Objections

Claims 11 and 18 are objected to because of the following informalities: Claim 18 is missing a comma and reads "CYP1ACYP2D6" in line 3 rather than "CYP1A, CYP2D6". Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-37 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claims are drawn to methods of measuring contaminants in water comprising introducing into a transgenic aquatic organism a DNA construct comprising one or more regulatory response element genes operably linked to a reporter gene, exposing the organism to a water sample to be

tested with conditions that permit expression of the reporter gene, detecting the reporter gene expression and correlating the expression to known standards to determine the quantity of contaminants in the water sample.

The specification has taught a number of regulatory response elements contained within an array of genes wherein the elements are crucial in activating gene expression in response to various contaminants in an aquatic environment including heavy metals, polyaromatic hydrocarbons, electrophile oxidants, endocrines and retinoids (paragraphs 0066-0073). The specification has taught a number of DNA constructs comprising upstream regions of various genes that comprise the regulatory response elements and can be operably linked to a reporter gene and used in zebrafish cultured cells in vitro to drive reporter gene expression in response to various contaminants (see legend, Figure 2, paragraph 0017). Figure 2 demonstrates that these constructs are responsive to corresponding contaminants in cells in vitro. The specification has contemplated making transgenic zebrafish whose genome comprises a vast array of reporter gene constructs that respond to various contaminants using the in vitro experiments as a starting point. The specification has taught expression of 4 constructs in transiently transfected zebrafish embryos without induction of gene expression by contaminants (Table 2, page 27). The specification, however, has only taught generating stable transgenic zebrafish whose genome comprises one of the small number of transgenes as listed in Table 2 on page 27 of the instant specification. The specification has taught that the transgene is expressed in transgenic zebrafish in the absence of contaminants (paragraph 0081).

The specification is not enabling for the claimed methods because the specification has failed to teach that the transgene constructs encompassed by the claims are responsive to

contaminants in vivo when stably incorporated into the genome of a fish or other aquatic organism. The specification has taught a number of constructs that are responsive to contaminants in vitro. The specification has taught using a small number of constructs comprising regulatory response elements to make transiently transfected zebrafish (see Table 2, page 26). However, the specification only teaches that the EF1-GFPZ-MTLCR was used to make stable transgenic zebrafish (paragraph 0081). The specification does not teach that the transiently transfected F_0 zebrafish made with any construct other than EF1-GFPZ-MTLCR maintained expression into adulthood or was transmitted through the germline, which would indicate stable incorporation into the genome.

The art at the time of filing held that the in vivo activity of a recombinant DNA construct in transgenic zebrafish is highly unpredictable and the in vitro activity cannot not be correlated predictably to the in vivo activity. Similarly, the activity in in vivo transient transfection assays cannot be correlated to expression or activity in stable transgenic fish. Transient transfection assays, both in vitro and in vivo, are such that multiple, unintegrated copies of a construct are present in the nucleus and are regulated differently when integrated in lower copy number into the genome as compared to extrachromosomal copies. Unintegrated transgenes are often misregulated and expressed ectopically in undesired tissues to varying levels (Sheets, 1998, Nature Biotechnology, Vol. 16, pages 233-234; Kroll and Amaya, 1996, Development, Vol. 122, pages 3173-3183, specifically page 3174, column 1, lines 9-12; Miller-Bertoglio, Chapter 3, specifically page 87, lines 8-12 and pages 102-103). This variability of transgene expression has a large impact on the predictability and reproducibility of phenotype, i.e. gene expression, in organisms resulting from transient transfection assays and must be considered in attempting to

generate stable transgenics. As set forth by Sheets, expression of unintegrated transgenes in transient transfection assays often results in ectopic and uncontrolled expression (page 233, col. 1, paragraph 3, lines 9-17). The instant specification has merely taught that the transgenes are expressed in transient transfected embryos and has not taught proper regulation of the genes in response to specific contaminants. The specification has not provided any teachings with regard to how to overcome the unpredictability set forth in the art of making transgenic zebrafish such that the skilled artisan can make the claimed aquatic organism for use in the claimed methods with a reasonable expectation of success.

The specification teaches using only one of the constructs used in the in vitro studies for the in vivo studies (AhRDtkluc3) and the responsiveness of that construct to contaminants in vivo was not demonstrated. The activity of no construct in stable transgenic zebrafish was demonstrated. Therefore, the specification does not allow the skilled artisan to make a correlation between the in vitro data shown in Figure 2 and the transgenic zebrafish of the claimed methods such that one of skill in the art could reliably predict that the claimed aquatic organism would be useful in the methods for detecting contaminants.

The specification is further not enabling for the full scope of the transgene constructs encompassed by the claims. The claims broadly encompass a transgene that merely comprises a regulatory response element from any of a vast array of genes, as listed in claim 11 for example, operably linked to a reporter gene. The specification teaches using large upstream regions from some of these genes wherein the regulatory response elements are contained within the large upstream regions (see paragraph 0017). The specification also teaches that these constructs contained additional minimal promoters. The claims do not require additional elements or any

DNA sequences to be present in addition to a regulatory response element and a reporter gene. The art at the time of filing held that the activity of recombinant DNA constructs made using the upstream regions of genes comprising the regulatory response elements of the instant invention was unpredictable. For instance, Carvan (1999, Mar. Biotechnol., Vol. 1, pages 155-166) taught that the 5' flanking region of the trout CYP1A3 gene that contains 5 AHREs amongst other transcription factor binding sites was less effective at driving luciferase expression in mouse cells in vitro than analogous constructs derived using mouse and human genes despite the promoters using the same molecular machinery (paragraph bridging pages 162-163). Carvan suggests that DNA regions flanking the response elements differentially affect the binding of species-specific transcription factors and thereby affects the overall activity of a recombinant gene. Therefore, the activity of a response element is dependent upon what flanking regions are or are not present in a transgene. Carvan also taught that the same elements have different activities in different cell types. Furthermore, Carvan (1999, Annals New York Academy of Sciences, Vol. 919, pages 133-147) and the instant specification have taught that transgenes are either removed or silenced in zebrafish, which can be prevented, at least in part, by including additional elements such as a locus control region to act as an insulator to stabilize gene expression. It would require undue experimentation for the skilled artisan to determine what sequences from the genes containing the regulatory response elements encompassed by the claims are necessary to make a transgene construct that is active and responsive to specific contaminants in a transgenic aquatic organism.

In view of the state of the art as it relates to the unpredictability of transgene activity, the breadth of the claims as it relates to the construction of the DNA constructs used in the claimed

methods, the lack of guidance with respect to the activity of the claimed transgenes and the lack of guidance in the specification with respect to what sequences to include in the constructs, it would require undue experimentation to implement the invention as claimed.

Claim Rejections - 35 USC § 112-2nd paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-37 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 and 2 are unclear because of the phrase "introducing into a transgenic aquatic organism" in line 3. It is unclear if a DNA construct is introduced into an organism that is already transgenic or if the introduction of the DNA makes the organism transgenic. Claims 3-10 and 13-37 depend directly or indirectly from claim 1. Claims 11 and 12 depend from claim 2.

Claims 1 and 2 are unclear because of the phrase "at least one regulatory response element gene". It is not clear what a "regulatory response element gene" is. A regulatory response element is an element that controls or regulates expression of a gene but is not a gene itself. It is unclear whether the claim is referring to an element or to a gene that is controlled by such an element. It is unclear if the entire regulatory element gene is operably linked to one reporter or multiple reporter genes or if regulatory elements from a gene are operably linked to one reporter or multiple reporter genes. The claims are wholly unclear. Claims 3-10 and 13-37 depend directly or indirectly from claim 1. Claims 11 and 12 depend from claim 2.

Claim 2, step a, is unclear. It is unclear if the step is referring to a single DNA construct having multiple regulatory response elements operatively linked to multiple reporter genes in tandem or if the step is referring to multiple separate constructs, each having a single regulatory element linked to a single reporter gene. Claims 11 and 12 depend from claim 2.

Claim 2 is unclear because of the phrase "the reporter genes". The phrase "the reporter gene" in line 5 refers to the actual coding sequence of the reporter gene. It is not clear is the phrase ""the reporter genes" in lines 12-13 is referring to the same gene as line 5, comprising just the coding sequence, or if it is referring to the entire DNA construct of step (a) including the regulatory elements. Claims 11 and 12 depend from claim 2.

Claims 3 and 4 recite the limitation "the regulatory response element" in line 1. There is insufficient antecedent basis for this limitation in the claim. Claim 1 refers to "the regulatory response element gene". Claims 5-10 and 13-37 depend from claim 4.

Claim 11 is unclear because it refers to "at least one response element from a gene selected from the group consisting of:"; however, the list of genes also contains elements such as AHRE1, AHRE2 and AHRE5, which are not genes. Claim 12 depends from claim 11.

Claims 11 and 18 are unclear because they contain abbreviated gene names. The full gene names cannot be determined based on the claim or the disclosure. For example, it is not clear if ACE1 is meant to represent acetylcholinesterase or angiotensin 1 converting enzyme. Both are abbreviated ACE1 in the art. Claim 12 depends form claim 11. Claims 19-37 depend from claim 18.

Claims 13-18 recite the limitation "the transgene" in line 1. There is insufficient antecedent basis for this limitation in the claim. Claims 19-37 depend from claim 18.

Claim 19 recites the limitation "the reporter element" in line 1. There is insufficient antecedent basis for this limitation in the claim.

Claim 34 is wholly unclear. Claim 34 recites the limitation "the native genes" in line 1. There is insufficient antecedent basis for this limitation in the claim. It is not clear what "the native genes" is referring to. Therefore, no clear interpretation of what the claim encompasses can be made.

Claim 34 recite the limitation "the transgenes" in line 1. There is insufficient antecedent basis for this limitation in the claim. It is unclear if the claim is referring to the entire reporter gene construct, the reporter gene, the promoter, or the response elements.

Claim 35 is unclear because it recited that the reporter gene has 85% homology to the "luciferase system". It is not clear how a gene can be homologous to a system.

Claim 36 is unclear because it states that the reporter gene has at least 85% homology to the species Aequorea. It is not clear how a gene can be homologous to a species.

Claim 36 is unclear because it refers to "Aequorea" as a species. "Aequorea" is a genus. A species names requires a genus and a specific epithet.

Conclusion

No claim is allowed.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Carvan, 2000, Annals of the New York Academy of Sciences. Toxicology for the new millennium, pages 133-147 was published October 2000, after the effective filing date of the instant application. The same or similar material was presented and publicly disclosed September 20-23 1999 at the Conference on Toxicology for the Next Millenium, Warrenton, VA, however, no published text is publicly available.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Valarie Bertoglio whose telephone number is (571) 272-0725. The examiner can normally be reached on Mon-Thurs 5:30-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Amy Nelson can be reached on (571) 272-0804. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Valarie Bertoglio
Examiner
Art Unit 1632

AU1637